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SYSTEMATIC EVALUATION OF *SISYRINCHIUM HITCHCOCKII* (IRIDACEAE): A RARE, ENDEMIC SPECIES OF WESTERN NORTH AMERICA

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ABSTRACT

Ambiguous characteristics used to separate sympatric *Sisyrinchium* species in western Oregon has generated concern among local botanists that *S. hitchcockii* Henderson, a rare, endemic grassland species, may not be a distinct entity. Furthermore, confusing keys and indiscernible herbarium specimens have hampered the verification of *S. hitchcockii* populations, leading to vague and imprecise species distribution, habitat association, and assessment of relative abundance. In this study we used a combination of morphological, molecular, and cytological analyses to determine whether *S. hitchcockii* is a distinct species. We found strong clade support for *S. hitchcockii* in a phylogenetic analysis, and differences in ploidy between *S. bellum* and S. *idahoense* var. *idahoense*, which are sympatric in west-central Oregon, suggest that they behave as separate biological species. The current distribution of *S. hitchcockii* appears to be fairly restricted and the taxon relatively rare, perhaps warranting formal conservation status by state and federal agencies.

KEY WORDS: *Sisyrinchium hitchcockii*, plant conservation, rare species, polyploid species.

Resolving rare species' taxonomic ambiguity and establishing clearly definable morphological traits for identification is important to understanding patterns of biogeography, endemism, evolution, and regional biodiversity, as well as the applied aspects of rare plant conservation. *Sisyrinchium hitchcockii* D. Henderson (Iridaceae), a rare and endemic species of western North America (ORBIC 2012), is a taxonomically confusing species with notable ambiguities surrounding its identification, distribution, and systematic placement. Historically restricted to Benton, Lane, and Douglas counties, Oregon, putative *S. hitchcockii* sightings and the few herbarium records indicate that it is rare, even when compared to co-occurring federally listed species (Gitzendanner & Soltis 2001; Giesler 2003; Severns et al. 2011). *Sisyrinchium hitchcockii* rarity might be due, in part, to difficulties distinguishing co-occurring *Sisyrinchium* species.

Two additional *Sisyrinchium* taxa, with more expansive geographical ranges, co-occur within the narrow distribution of *S. hitchcockii*. *Sisyrinchium bellum* Watson is found from southwestern

Washington, south through western Oregon to southern California. *Sisyrinchium idahoense* var. *idahoense* (Bicknell) D. Henderson occurs from the southwestern corner of British Columbia south to northern California and eastward into northern Idaho and Montana.

Sisyrinchium hitchcockii was originally described as an octoploid species (n=32) and is broadly sympatric with the tetraploid (n=16) *S. bellum* (Henderson 1976; Cholewa & Henderson 2002) and *S. idahoense* var. *idahoense*, which varies from octoploid to duodecaploid. In his original description of *S. hitchcockii*, Henderson (1976) considered an elongate rhizome to be diagnostic for the species. However, authors of recent floras (e.g., Kozloff 2005) have elected not to use this character, likely because most herbarium specimens lack a rhizome (Meyers pers. obs.). In live specimens, *S. bellum* differs from *S. hitchcockii* and *S. idahoense* var. *idahoense* by two-toned tepals. But in keys, *S. hitchcockii* and *S. idahoense* var. *idahoense* have overlapping floral traits such as leaf width, perianth length, and perianth color (Henderson 1976; Kozloff, 2005). Moreover, *Sisyrinchium* species tend to make poor herbarium specimens because during the drying process the flowers usually deform. Retrospective classification and construction of a reliable and useful *Sisyrinchium* key from preserved material is almost impossible.

Because *Sisyrinchium hitchcockii* floral characteristics have been presented ambiguously, local botanists and land managers have questioned the validity of the species. We used DNA sequencing, flow cytometry, and morphological characters in a phylogenetic and taxonomic framework to determine whether *S. hitchcockii* is a species distinct from related congeners. We also provide a key without the use of rhizome traits to identify *Sisyrinchium* taxa within the historic range of *S. hitchcockii*.

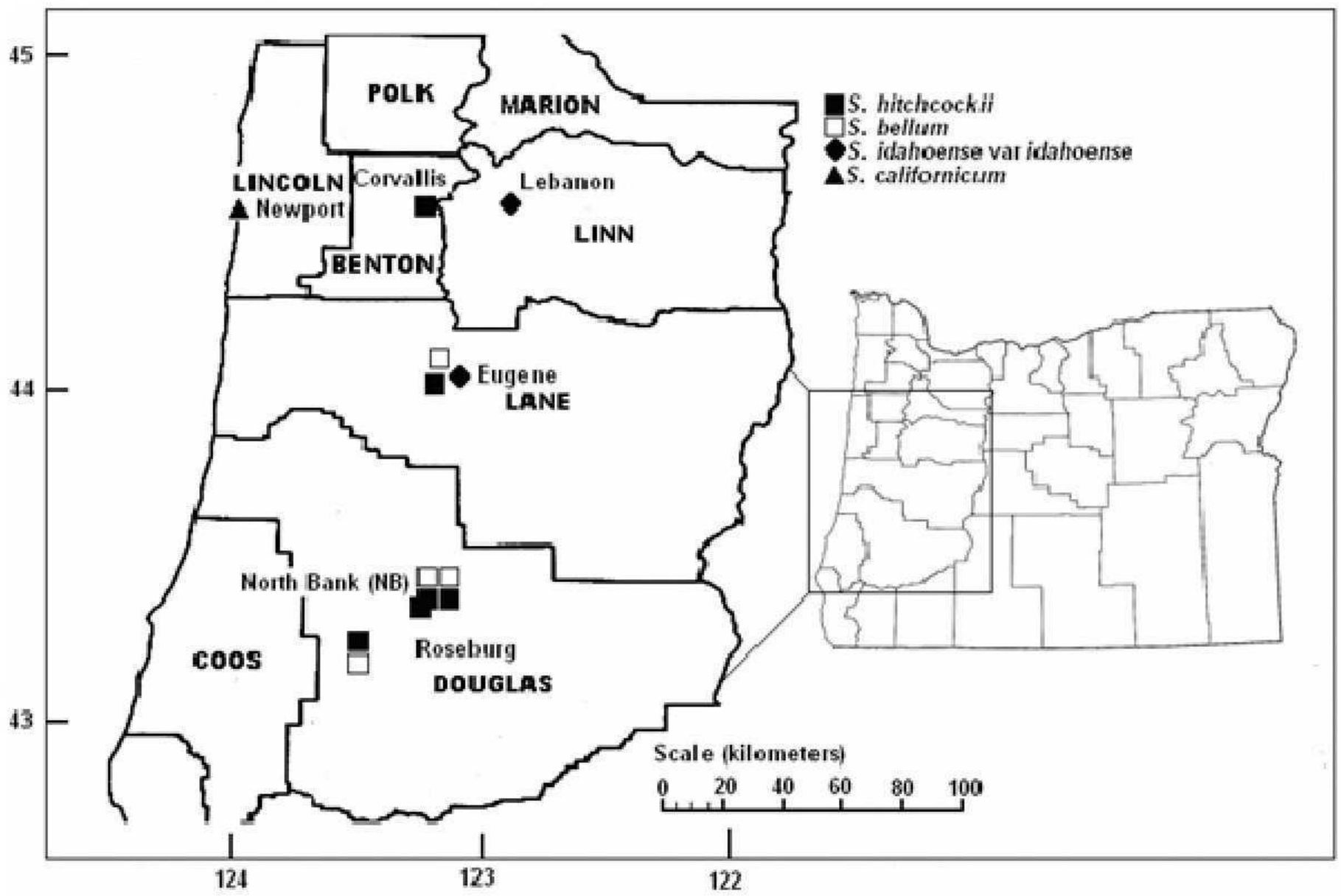

Figure 1. Locations of *Sisyrinchium* populations surveyed. Morphological data was taken from all sites. Material for the DNA and flow cytometry analyses were collected from all sites with the exception of *S. bellum* and *S. hitchcockii* from the area of Eugene, Oregon.

Table 1. Morphological characters surveyed from live and herbarium specimens. Bold characters indicate those that reliably distinguish *S. hitchcockii*, *S. bellum* and *S. idahoense var. idahoense* from one another. *Indicates traits not always measureable on herbarium specimens.

Characters measured for each species	Sample size (n)	25%	Median	75%
S. hitchcockii				
Perianth length (mm)	**100**	**14**	**15**	**17**
Perianth width (mm)*	72	4.5	5	5
Perianth length/width ratio*	**72**	**2.98**	**3.2**	**3.6**
Plant height (cm)*	64	26.88	31.5	37.5
Stem width (mm)	64	2	2	3
Leaf width (mm)*	40	2	2.25	3
Filament column length (mm)	**64**	**8**	**8**	**8.5**
Yellow eye length (mm)*	**64**	**0**	**1**	**1.5**
Outer bract length (mm)	64	24	27.5	34.25
Inner bract length (mm)	64	19	21.5	23.25
Bract outer/inner ratio	64	1.08	1.32	1.68
S. bellum				
Perianth length (mm)	**72**	**10**	**10**	**11**
Perianth width (mm)*	39	4	5	5
Perianth length/width ratio*	**39**	**2**	**2.2**	**2.4**
Plant height (cm)*	62	22.13	26.75	30
Stem width (mm)	62	2	2	2
Leaf width (mm)*	48	2	2.75	3
Filament column length (mm)	**43**	**5**	**5**	**5.5**
Yellow eye length (mm)*	**43**	**2**	**2.2**	**2.4**
Outer bract length (mm)	43	20	25	28.5
Inner bract length (mm)	43	19	21	23
Bract outer/inner ratio	43	1.04	1.11	1.30
S. idahoense var. idahoense				
Perianth length (mm)	**54**	**12**	**13**	**14**
Perianth width (mm)*	24	4	4	5
Perianth length/width ratio*	**24**	**2.95**	**3.25**	**3.5**
Plant height (cm)*	44	24	30	34
Stem width (mm)	44	1.5	2	2
Leaf width (mm)*	39	2	2	2.75
Filament column length (mm)	**40**	**4**	**5**	**5.13**
Yellow eye length (mm)*	**40**	**2**	**2**	**2.5**
Outer bract length (mm)	40	29	34	43.25
Inner bract length (mm)	40	21.75	24.5	26
Bract outer/inner ratio	40	1.23	1.48	1.7

MATERIALS AND METHODS

Locations of putative *Sisyrinchium hitchcockii* populations were obtained from the herbarium at Oregon State University and the Oregon Flora Project website (OFP 2012). With the exception of one putatively disjunct *S. hitchcockii* location (*Tracy 15947*, WTU) from Humboldt County, California, all sites were visited between May 15 and May 30, 2009. In total four extant populations of *S. hitchcockii* were found. Locations of *S. bellum* and *S. idahoense* var. *idahoense*, nearby or sympatric with each extant *S. hitchcockii* population, were located using the same methodology (Fig. 1).

Morphological analysis. For each plant we measured 11 morphological traits on both live and herbarium specimens, although on herbarium specimens some of the traits could not be reliably measured (Table 1). Tepal length and width measurements were taken from outer tepals and measured to the tip of the awn from the base of the tepal and at the widest tepal width. Yellow eye measurements were taken from the center of the flower to the tip of the yellow coloration. Filament column measurements were taken from the base of the fused filaments to the tip of the anthers. Stem and leaf width were measured from the widest portion of the stems and leaves. Plant height was measured from the ground to the base of the highest inflorescence. Outer and inner spathe bracts enclosing the inflorescences were measured from point where both bracts meet the stem to the tip of each bract.

Phylogenetic analysis. Fresh leaf tissue was collected from four individuals of *Sisyrinchium hitchcockii*, three individuals of *S. bellum*, and two individuals of *S. idahoense* var. *idahoense* from sympatric and allopatric populations of each species (Fig. 1). One specimen of *S. californicum* (Ker Gawler) Dryander was also collected and sequenced to serve as an outgroup. Vouchers have been placed in the Oregon State University Herbarium (OSC). The nuclear ribosomal internal transcribed spacer (nrITS) was amplified following the protocol of Karst (2007). PCR products were purified using QIAquick PCR purification kits (Qiagen) and Sanger sequencing was performed by Center for Genome Research and Biocomputing at Oregon State University (Corvallis). Sequences were aligned "by eye" with BioEdit for Windows 95/98 (Hall, 1999) and the trimmed ~700 bp regions were deposited in GenBank (accession numbers HQ414469-HQ414478). Phylogenetic analyses were conducted using PAUP* ver. 4b10 (Swofford 2002). The most parsimonious tree was found using a branch and bound maximum parsimony search under the furthest addition sequence setting with MulTrees on. Gaps were scored as missing data. Branch support values were calculated using 1000 bootstrap replicates.

Ploidy measurements. Flow cytometry was performed on fresh leaf material from four individuals of *Sisyrinchium hitchcockii*), three individuals of *S. bellum*, and two individuals of *S. idahoense* var. *idahoense*, using a Partec GmbH ploidy analyzer. *Sisyrinchium bellum* was used as an internal standard because it is not known to vary in chromosome number (Henderson 1976; Goldblatt & Johnson 1990; Cholewa & Henderson 2002).

RESULTS

Within the single most parsimonious branch and bound phylogenetic tree, all specimens of *Sisyrinchium hitchcockii*, *S. bellum*, and *S. idahoense* var. *idahoense* formed monophyletic clades (Fig. 2).

Of the 11 traits surveyed (including allometric ratios), four traits were found (tepal length, tepal length to width ratio, filament column length, and center yellow eye width) that consistently distinguished *Sisyrinchium hitchcockii*, *S. bellum*, and *S. idahoense* var. *idahoense* from one another.

Flow cytometry revealed that all *Sisyrinchium hitchcockii* samples produced peaks in a channel twice the height of all *S. bellum* samples, indicating that all *S. hitchcockii* samples surveyed were octoploid. Additionally, all *S. idahoense* var. *idahoense* samples produced peaks in a channel three times the height of *S. bellum* samples, indicating that all *S. idahoense* var. *idahoense* samples surveyed were duodecaploid.

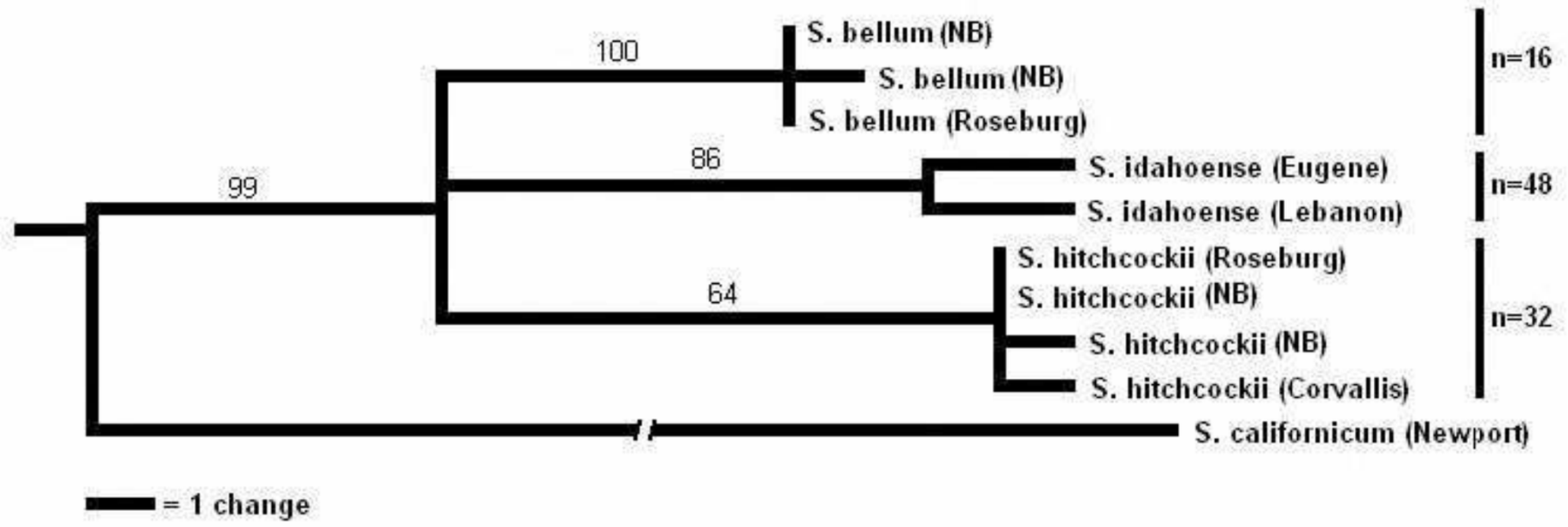

Figure 2. The single most parsimonious tree found for the nrITS dataset. Numbers to the right indicate chromosome numbers. NB = Bureau of Land Management North Bank Habitat Management Area.

DISCUSSION

Based on the molecular phylogenetic tree constructed in this study (Fig. 2), the collections of *Sisyrinchium hitchcockii* we genotyped form a well-defined monophyletic clade separate and distinct from both *S. bellum* and *S. idahoense* var. *idahoense*. If *S. hitchcockii* were conspecific with either *S. bellum* or *S. idahoense* var. *idahoense*, it is likely that one or more specimens of *S. hitchcockii* would have occurred within either the *S. bellum* or *S. idahoense* var. *idahoense* clades.

Further support for the validity of *Sisyrinchium hitchcockii* is indicated by the chromosome numbers of the specimens we sampled. Although Henderson (1976) and Cholewa and Henderson (2002) reported that most *S. idahoense* var. *idahoense* populations and all *S. hitchcockii* within western North America are octoploid, all samples of *S. idahoense* var. *idahoense* we surveyed within western Oregon were duodecaploid. It should be noted, however, that prior to this study, no chromosome number counts had been recorded from *S. idahoense* var. *idahoense* specimens from western Oregon; rather all reports have been from populations in eastern Oregon, Idaho, Montana, and British Columbia.

Although greater sampling is warranted, our results suggest that all or most *Sisyrinchium idahoense* var. *idahoense* populations in western Oregon are duodecaploid and therefore reproductively isolated from octoploid populations of *S. hitchcockii*. Furthermore, because *S. hitchcockii* and *S. idahoense* var. *idahoense* are putative sister species (Karst 2007), and because of the limited geographic range of *S. hitchcockii*, we hypothesize that *S. hitchcockii* may have diverged from local *S. idahoense* var. *idahoense* populations as a result of ploidy reduction.

Previously published keys have failed to utilize floral diagnostic characters to separate these species. Therefore, we have constructed what we consider a more functional and pragmatic key to *Sisyrinchium* species in western Oregon:

1. Outer perianth segments < 2.5 times longer than wide and < 13 mm long; underside of tepals a lighter color than the top side ..... **Sisyrinchium bellum**
1. Length to width ratio of outer perianth segments > 2.5, perianth segments typically > 13 mm long and up to 21 mm long; underside of tepals the same color as the top side.

    2. Filament column dark purple and > 6 mm; dark purple near the center of the flower, yellow eye of flower small or absent ..... **Sisyrinchium hitchcockii**
    2. Filament column yellow-tan to tan-purple and < 6 mm long; yellow eye of flower conspicuous ..... **Sisyrinchium idahoense** var. **idahoense**

While this key is functional for identifying western Oregon *Sisyrinchium* species in the field, users may still experience difficulty identifying herbarium specimens due to the poor drying characteristics of *Sisyrinchium* species.

Although we could not unambiguously demonstrate that *Sisyrinchium hitchcockii* does not currently exist at other sites in western Oregon or northern California, it currently appears that the species may be restricted to a handful of sites in Douglas, Lane, and Benton counties, Oregon (Fig. 1). Historical collections suggest that *S. hitchcockii* may have had a broader range (OFP 2012), but its current distribution appears to be restricted from habitat loss, degradation, and succession over the last several decades. Considering the restricted distribution of *S. hitchcockii*, relative isolation of known populations, and the presence of continued threats to this species' existence, we recommend consideration of *S. hitchcockii* for conservation protection status by state and federal agencies.

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